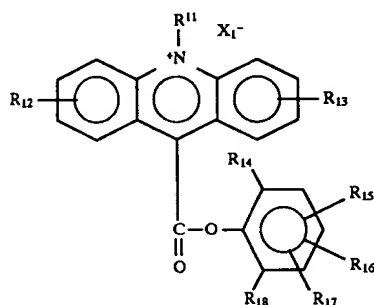


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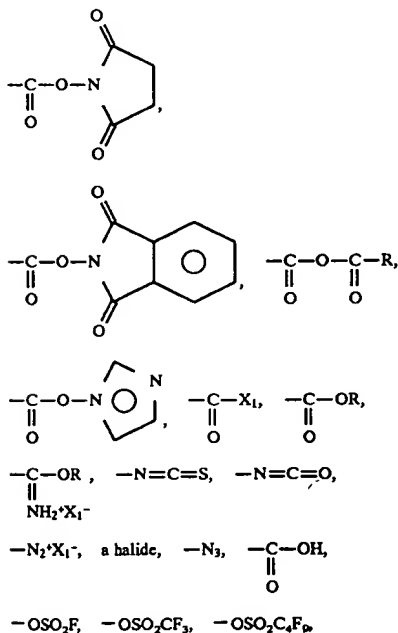
superoxide anion to an acridinium derivative, so it is not necessary to use strongly alkaline conditions as in the conventional methods. The reaction can be carried out around the neutral point to generate strong luminescence which is stable over a long period of time. Further, when an acridinium derivative is used as a label and a superoxide anion is made to act on the label around the neutral point, it is possible to obtain a strong luminescence which is stable over a long period of time. Thus, it is possible to provide a method of immunoassay which is accurate and has a high precision.

We claim:

1. A method for emitting chemiluminescence from an acridinium ester in an immunoassay, a hybridization assay or an immunoblot assay, wherein said ester is a compound of the formula (2):

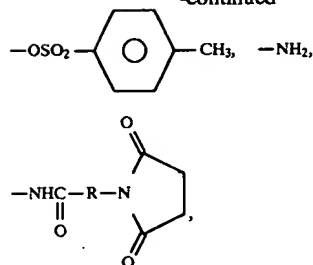


wherein  $R_{11}$  is an alkyl, alkenyl, alkynyl or aryl group;  $R_{12}$ ,  $R_{13}$ ,  $R_{15}$  and  $R_{17}$  are independently a hydrogen atom, halide atom, amino, carboxyl, hydroxyl, alkoxy or nitro group;  $R_{14}$  and  $R_{18}$  are independently an alkyl, alkenyl, alkynyl, aryl, alkoxy, amino, amido, sulfonamido or sulfide group;  $R_{16}$  is  $-R_{19}-R_{10}$ ;  $R_{19}$  is not required but optionally can be an alkyl, aryl or aralkyl group;  $R_{10}$  is selected from the group consisting of:

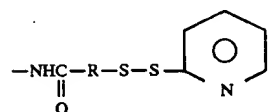


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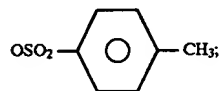
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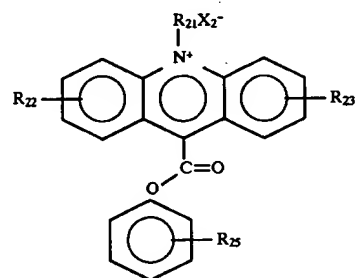
and



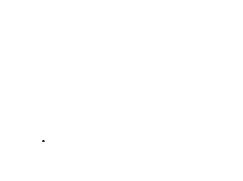
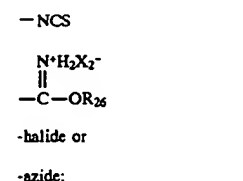
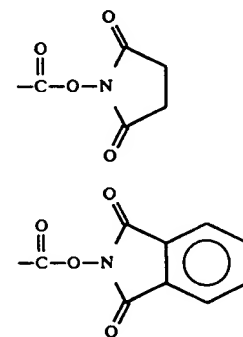
$X_1$  is  $CH_3SO_3^-$ ,  $OSO_2F^-$ , a halide,  $OSO_2CF_3^-$ ,  $OSO_2C_4F_9^-$ , or



and R is an alkyl, aryl or aralkyl group; or said ester is a compound of the formula (3a):



wherein  $R_{25}$  comprises one of the following:



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$R_{21}$  and  $R_{26}$  are independently a hydrogen atom or  $C_1-C_{10}$  optionally substituted alkyl, alkenyl, alkynyl or aryl group;  $R_{22}$  and  $R_{23}$  are independently a hydrogen atom, halide atom, amino, substituted amino, carboxyl, hydroxyl, alkoxy or nitro group; and  $X_2$  is a halide atom, comprising reacting at a pH of 6 to 10 said acridinium ester and a superoxide anion produced in situ by electrochemical reduction of oxygen dissolved in an electrolyte using electrodes in the presence of a flavin compound, wherein said acridinium ester is attached as a label to a reagent in said immunoassay, hybridization assay or immunoblot assay.

2. The method of claim 1, wherein the electrochemical reduction is performed in the presence of  $1 \times 10^{-9}M$  to  $1 \times 10^{-4}M$  of the flavin compound at a potential of  $-0.3$  to  $-0.7V$  (vs.  $Ag/AgCl$ ).

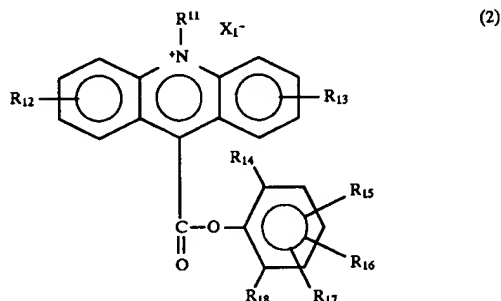
3. The method of claim 1 wherein the electrochemical reduction comprises use of an electrode comprising said flavin compound.

4. The method of claim 1, wherein the acridinium ester is 4-[2-succinimidyloxycarbonyl]ethyl]phenyl-10-methylacridinium-9-carboxylate fluorosulfate.

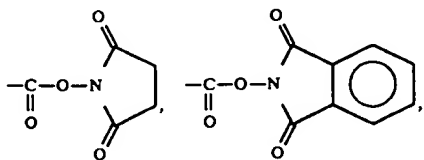
5. A method of detecting a substance to be examined in a sample in an immunoassay, hybridization assay or immunoblot assay, comprising,

binding a chemiluminescent labeled substance having affinity for said substance to be examined with said substance to be examined in a sample to produce a complex of said substance to be examined and said chemiluminescent labelled substance having affinity therefor; separating said complex;

reacting said chemiluminescent label with a superoxide anion at a pH of 6 to 10, said label being an acridinium ester of the formula (2):

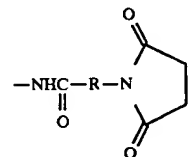
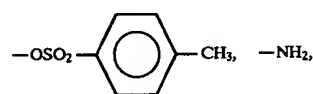
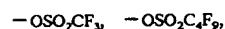
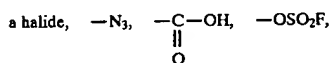
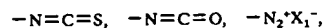
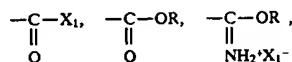
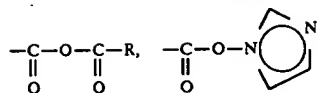


wherein  $R_{11}$  is an alkyl, alkenyl, alkynyl or aryl group;  $R_{12}$ ,  $R_{13}$ ,  $R_{15}$  and  $R_{17}$  are independently a hydrogen atom, halide atom, amino, carboxyl, hydroxyl, alkoxy or nitro group;  $R_{14}$  and  $R_{18}$  are independently an alkyl, alkenyl, alkynyl, aryl, alkoxy, amino, amido, sulfonamido or sulfide group;  $R_{16}$  is  $-R_{19}-R_{20}$ ;  $R_{19}$  is not required but optionally can be an alkyl, aryl or aralkyl group;  $R_{20}$  is selected from the group consisting of:

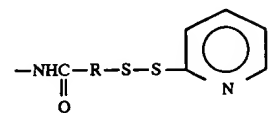


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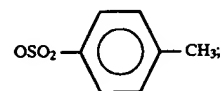
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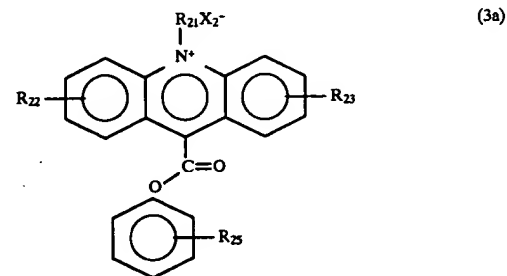
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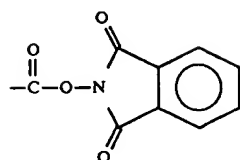
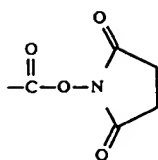


$X_1$  is  $CH_3SO_4-$ ,  $OSO_2F-$ , a halide,  $OSO_2CF_3-$ ,  $OSO_2C_4F_9-$ , or



and  $R$  is an alkyl, aryl or aralkyl group; or said label is an acridinium ester of the formula (3a):





-NCS



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- (a) -halide or (e)  
-azide; (f)
- 5  $R_{21}$  and  $R_{26}$  are independently a hydrogen atom or  $C_1-C_{10}$  optionally substituted alkyl, alkenyl, alkynyl or aryl group;  $R_{22}$  and  $R_{23}$  are independently a hydrogen atom, halide atom, amino, substituted amino, carboxyl, hydroxyl, alkoxyl or nitro group; and  $X_2$  is a halide atom; wherein said superoxide anion is produced in situ by electrochemical reduction of oxygen dissolved in an electrolyte using electrodes in the presence of a flavin compound; and
- (b) 10 measuring luminescence of said chemiluminescent label to detect said substance to be examined.
- 15 6. The method of claim 5 wherein said binding step in said hybridization assay is reaction between a nucleic acid and a nucleic acid complementary thereto.
- (c) 20 [7. The method of claim 5, wherein the acridinium ester is 4-[2-(succinimzyloxy/carbonyl)ethyl]phenyl-10-methylacridinium-9-carboxylate fluorosulfate.]
- (d)

\* \* \* \* \*

add  
a1